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AN ALKALINE THIONIN METHOD OF STAINING RICKETTSIAE[1]

BY JAMES CRAIGIE[2]

Abstract

The development of an alkaline thionin stain for rickettsiae in yolk sac preparations is described. This stain consists of a balanced mixture of thionin, methylene blue, sodium carbonate, and sodium hydroxide. The methylene blue prevents precipitation of the base of thionin. The sodium carbonate serves to enhance the staining of rickettsiae and to suppress the staining of cells and yolk granules. The formation of thiazin carbonate is checked by the presence of sodium hydroxide. There is some evidence that this method may be of value for the staining of the larger viruses and spirochaetes.

Macchiavello's method (7, p. 896) stains rickettsiae red against a contrasting blue cytoplasmic background and is unrivalled for routine examination and comparison of yolk sac or tissue culture preparations. Nevertheless, this method appears to have some limitations that may be important in special studies. In the course of investigation of the variation and early development of *Rickettsia prowazeki* in developing eggs it recently became evident that Macchiavello's method was not entirely reliable for the detection of minute coccoid forms (150 to 200 mμ) or sparsely distributed slender bacillary forms. In infected eggs in the third week of development larger coccoid bodies of doubtful nature (up to 300 mμ) were sometimes encountered. Differentiation, in such instances, was found to be very critical. Because of these difficulties an attempt was made to find a selective, progressive method of staining in which the hazards of timing and differentiation would be eliminated. Previous experience with Giemsa stain and with the azures suggested that some member of the thiazin series might have the desired characteristics.

A number of azure preparations were made from methylene blue, according to the bichromate–sulphuric-acid method advocated by Lillie (4, 5). Some of these preparations had been over-neutralized with sodium carbonate and these seemed to give better results than the others. It was noted that if carbonate, to a concentration of 5 to 10%, was added to the dye immediately before use, the staining of yolk granules was reduced. Further, if a dilute alkaline azure solution was brought to boiling point it became dichroic in a few seconds, i.e., the colour of the image of an electric lamp filament viewed

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Contribution from the Connaught Laboratories, University of Toronto, Toronto, Ont. with financial assistance from the National Research Council of Canada. A description of the method was submitted in Project Med. 8, Memorandum No. 8 (May 17, 1944).

[2] *Research Member, Connaught Laboratories and Associate Professor of Virus Infections, School of Hygiene, University of Toronto.*

through the dye solution changed from blue to violet or garnet. Very selective staining of rickettsiae in yolk sac smears was obtained when such heated alkaline mixtures were used. The staining of the yolk granules was virtually suppressed, the cytoplasmic structure of the columnar cells was lightly stained, whereas the rickettsiae were coloured a deep violet.

The change induced on heating the alkaline azure solution was considered to be unduly complicated, consisting in part of oxidation and in part of formation of dye base that precipitated within a short time. Because of this precipitation, the heated mixture had to be used immediately. In order to circumvent the inconvenience of heating small quantities of stain immediately before use, larger quantities were prepared, heated with carbonate, and filtered. The precipitate was then extracted with ethyl or methyl alcohol containing 1% hydrochloric acid. The extracts were dried and redissolved in the minimum volume of alcohol. Ethylene dichloride was sometimes used as a solvent for the dye base, which was then recovered as the chloride. Heating in the presence of alkali merely served to effect a further removal of methyl groups from the crude thiazin mixtures (3) and to secure preparations containing a greater proportion of the lower homologues. The order of solubility of the thiazin bases is given by Haynes (1, 2) as follows:

Tetraethyl thionin
Tetramethyl thionin (methylene blue)
Trimethyl thionin (azure *B*)
Diethyl thionin
Dimethyl thionin (azure *A*)
Monomethyl thionin (azure *C*)
Thionin

The preparations of lower homologues obtained from the crude azure mixtures by heating with carbonate were tested in solution with 5% carbonate for staining properties. The results were similar to those obtained with the crude azures that had been heated with alkali immediately before use. Exact balance of the concentration of dye and carbonate, however, was found to be important. If too much alkali was added, rapid precipitation of the dye occurred; if too little was added, the carbonate of the dye was rapidly formed when the solution was exposed to the air.

The best results in this phase of the investigation were obtained with a preparation having an absorption maximum of 606 mμ. It was found that the optimum amount of carbonate for this sample was that which produced the maximum colour change from blue to lilac-pink. If slightly less carbonate was added, the mixture, when exposed to the air on slides, changed from pink to blue. A similar and instantaneous colour change was produced when the dilute mixture was shaken in a tube to which a little carbon dioxide had been added. A series of tests showed that the thiazin carbonates formed on exposure to air or carbon dioxide had no value as stains, being rapidly eluted when the smear was washed with acid, neutral, or alkaline distilled water. This experience suggests that some of the more obscure vagaries of Giemsa or

other thiazin–eosinate preparations, which cannot be corrected by phosphate buffer, may be due to absorption of carbon dioxide and formation of thiazin carbonate.

Table I summarizes the results obtained when sodium carbonate (5%) was added to dilute solutions (0.001%) of certified samples of dyes of the thiazin series. It will be noted that the most intense staining of rickettsiae was obtained with azure *C*, which is relatively unstable in alkaline solution. At the very alkaline reaction employed, the staining of tissue cells was less marked than that obtained at pH 8 to 9 (see 1, 2).

TABLE I

THE STABILITY OF THIAZIN DYES IN SODIUM CARBONATE SOLUTION

Dye (National Aniline & Chemical Co., Inc.)	Certification number	No. of methyl groups	Effect of 5% sodium carbonate		
			Colour change	Stability	Staining value for rickettsiae
Methylene blue (chloride)	*NA* 19	4	None	Relatively stable	±
Azure *B* (bromide)	Lot 9610	3	None	Stable for some hours	+
Azure *A* (chloride)	*NAs* 9	2	Red or pink	Precipitation in one hour	++
Azure *C* (chloride)	*NAc* 3	1	Red or pink	Precipitation in 15 to 20 min.	+++
Thionin	*NT* 12	0	Red or pink	Immediate precipitation	Nil

Methylene violet (Lots *NLv* 2 and *NLv* 4) was included in this investigation, but it was necessary to use these in combination with the higher homologues of the series. Methylene blue has a solvent effect on the water insoluble methylene violet (6) and the azures have a similar action. It was concluded that methylene violet, present in small amounts in the experimental lots of azure prepared by bichromating methylene blue, was not a factor in the selective staining of rickettsiae.

Although the commercial sample of azure *C* gave fairly satisfactory results in alkaline solution, these were not quite comparable to those obtained with the experimental lots of azure *C*. The commercial sample showed little polychrome effect. Its stability in alkaline solution was unsatisfactory and it stained rickettsiae less intensely.

The low absorption maximum and the polychrome tendencies of the best experimental lot of azure *C* finally suggested that thionin might be an important constituent of this lot. Previous tests (see Table I) showed that thionin alone was too unstable in alkaline solution. The base precipitated almost immediately after the addition of alkali, and at best only a faint tinting of the smears was obtained. Because methylene violet is soluble in an aqueous solution of methylene blue, a series of trials were made in which the other

thiazin dyes were added to the thionin solution before it was mixed with carbonate.

The most promising results were obtained with methylene blue; and a subsequent quantitative study defined the optimum proportions of methylene blue and thionin. This was found to be one part of methylene blue to four parts of thionin. Since carbonate alone afforded no protection against the formation of dye carbonate, a mixture of sodium carbonate and sodium hydroxide was adopted. Finally, the optimum proportion and concentration of the dye and the alkaline solution were determined.

Throughout these investigations with very alkaline dye solutions, the test smears had been fixed in formalin with the idea of counteracting the detergent effect of the alkali on the smears. When a suitably balanced staining mixture had been developed, various methods of fixation were compared. It was found that formalin fixation was essential to proper staining.

The method of staining rickettsiae, which will now be described in detail, has yielded consistent results with fresh yolk sac and other embryo preparations. When yolk sac emulsions have been kept for some time with formol saline, the yolk granules stain more readily than in fresh preparations. Modifications of this stain have yet to be worked out for fixed, paraffin-embedded sections. The method has given good results when used for the rapid staining of vaccinia elementary bodies and spirochaetes and would appear to be worthy of a trial for psittacosis, lymphogranuloma, and other large viruses. This thionin method also stains bacteria intensely. If applied to preparations of *Bacillus proteus OX*19 after tannic-acid–ferric-chloride mordant, good flagellar staining is obtained.

Method

(1) Prepare yolk sac or tissue smears in the usual way and allow them to dry.
(2) Fix with gentle heat.
(3) Immerse slide in 10% formalin in distilled water for 5 to 10 min.
(4) Remove slide and rinse with distilled water to which sufficient sodium hydroxide has been added to make it alkaline to thymol blue.
(5) Apply a freshly made mixture of equal parts of the following stock solutions:

A.	0.1% thionin (aqueous)*	4 cc.
	0.1% methylene blue (aqueous)	1 cc.
	Distilled water	45 cc.
B.	*N*/1 sodium hydroxide	6 cc.
	10% sodium carbonate (aqueous)	44 cc.

Allow staining to proceed for 10 min. for membrane or tissue smears and for 15 min. for yolk sac smears.

(6) Rinse thoroughly with distilled water (neutral or slightly acid).

** Prepare the 0.1% thionin solution by grinding the dye in a mortar with distilled water; then make up to the required volume and heat to the boiling point. Allow the solution to stand overnight and decant gently into a stock bottle. Do not filter.*

(7) Dry rapidly without heating. (An atomizer rubber bulb fitted with a glass nozzle furnishes a convenient means of providing a blast of air to blow off excess water and promote rapid drying.)

It is probable that the selective staining of rickettsiae obtained by this method is due to adsorption of the base of thionin. In fact, it was on this hypothesis that the method was elaborated. The methylene blue acts as a solvent for the thionin base, which would otherwise be precipitated on addition of the alkaline solution. This alkaline solution has three functions:

(*a*) to inhibit the staining of yolk granules and reduce the staining of cells,

(*b*) to form the base of thionin, and

(*c*) to combine with carbon dioxide absorbed from the atmosphere while staining is in progress and thus prevent the formation of thiazin carbonate.

It is a simple matter to adjust the staining mixtures for different samples of dye or for a hydroxide solution that has become partially converted to carbonate. Various adjustments of this sort are suggested in Table II. The proportions of thionin and methylene blue recommended are those found to be most suitable for routine use. More intense staining of very minute rickettsiae or vaccine virus will be obtained if the concentration of thionin is increased.

TABLE II

ADJUSTMENT OF ALKALINE THIONIN MIXTURES FOR OPTIMUM STAINING OF RICKETTSIAE

Defect in staining solution or in stained smear	Adjustment required
Smear too uniform a blue; rickettsiae too blue	Increase the proportion of thionin
Visible precipitate in the staining mixture, or the presence of thionin crystals on the stained smear	Increase the proportion of methylene blue
Too intense staining after 10 minutes	Reduce the concentration of the methylene-blue–thionin solution
Stain on the slide turns blue at the margin in 10 minutes*	The sodium hydroxide has been converted to carbonate. Prepare a fresh solution
Rickettsiae not deeply stained	Increase staining time or reduce the proportion of alkaline solution (*B*) to dye solution (*A*)

* *A gross excess of carbon dioxide in the air will also produce this effect.*

The thionin method that has been described is recommended for use under conditions where Macchiavello's stain does not yield satisfactory results, or a supplementary stain is desired. Like Macchiavello's stain, it is limited in its use to film and impression smear preparations. With these it yields results somewhat similar to those obtained by Giemsa methods and is simpler to apply than the latter.

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CHEMOTHERAPY IN EXPERIMENTAL TUBERCULOSIS[1]

By K. I. Melville[2] and R. L. Stehle[3]

Abstract

Seventy-nine compounds comprising 22 *p*-aminobenzene derivatives, 10 *o*-aminobenzene derivatives, 11 *m*-aminobenzene derivatives, 8 *p*-N-ethylaminobenzene derivatives, 10 isomeric hydroxychloroanilines, 3 diaminodiphenylsulphones and 15 miscellaneous agents, have been compared for their effects upon the course of experimental tuberculosis in guinea pigs inoculated intraperitoneally with virulent human tubercle bacillus (Strain *H* 37 *R.V.*). Sixty-five of these compounds gave entirely negative results. On the other hand, 14 of the agents tested, namely, *p*-aminophenol, *p*-ethylaniline, *p*-chloroaniline, *p*-aminophenyl hexyl ether, ethyl-*p*-aminobenzoate, 2,4-dichloroaniline, *p*-N-ethylaminophenol, 3-chloro-4-hydroxyaniline, 2-chloro-4-hydroxyaniline, 2-chloro-5-hydroxyaniline, 2-hydroxy-3-chloroaniline, promin, rodilone, and sulphathiazole led, in a number of different experiments, to varying degrees of prolongation of the survival time of some of the animals treated with them, in comparison with both untreated controls and animals treated with other agents. The average survival times of all the animals treated with these agents were also prolonged in several different series of experiments in which each of these agents was tested. None of the latter agents led to a curative effect and all animals both treated and untreated, however long they survived, showed at autopsy gross evidence of tuberculosis involving spleen, liver, lungs, and glands. Finally, it must be emphasized that none of these compounds offer any promise as a cure for tuberculosis, but the results described would suggest that further investigation of chemical agents related to these substances might be worthwhile.

Considering its fundamental importance the problem of finding an effective chemotherapeutic agent for tuberculosis has not been extensively investigated. The earlier favourable results obtained by Ehrlich in the chemotherapy of syphilis and the success that has been attained following the work of Domagk on the chemotherapy of streptococcic infections leave little doubt that, with continued systematic investigations of chemical agents in experimental tuberculosis, a solution to this problem will one day be found.

The object of this paper is to record some data that were obtained in this laboratory, in connection with this problem.

From time to time, smaller or larger groups of chemicals have been tested by different investigators for their effects in experimental tuberculosis, but none of these have led to any indication as to what class of agents might be most advantageously selected for study. A large number of the studies on the problem deal primarily with the effects of chemicals on the growth of the tubercle bacillus *in vitro*, on the assumption that a highly bacteriostatic or bactericidal activity *in vitro* might offer a lead for *in vivo* experiments. Experience has shown, however, that this is not necessarily the case, and

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[2] *Associate Professor of Pharmacology, McGill University.*
[3] *Professor of Pharmacology, McGill University.*

indeed, most of the highly effective chemotherapeutic agents used in other diseases exert either no action at all or only a questionable action *in vitro*. Thus it is evident that the chemotherapeutic value of any agent can only be assessed by testing its effects upon the course of the disease *in vivo*.

As is well recognized there are many difficulties involved in such a procedure, owing both to the variability of the course of the experimentally induced disease, and to the considerable time required if the agent is to be tested in a chronic infection such as is characteristic of tuberculosis in man.

Historical

In 1913 DeWitt (6) investigated the *in vitro* activity of several aniline dyes against virulent human tubercle bacilli and found some of these to exert a definite action in high dilutions. Studying these agents *in vivo*, however, she (7) could obtain "no favorable or curative influence in experimental tuberculosis in guinea pigs." Lewis has reported similar findings with a large number of aniline dyes studied both *in vitro* (21) and *in vivo* (22).

DeWitt (8, 9) later investigated a series of organic mercury compounds for their effects in experimental tuberculosis; among these were some substituted aniline mercury derivatives. For comparison a number of corresponding non-mercurated aniline compounds were similarly tested. Among the latter were para-, ortho-, and meta-nitroaniline. Several of the mercurated agents showed some bactericidal activity *in vitro* in high dilutions, and two of these compounds, the bis-sodium nitrophenolate of mercury and the para-N-ethyl-aminonitrobenzene mercury acetate, appeared "in slow progressive chronic tuberculosis to bring about a definite fibrosis and healing of tubercles in different organs such as is not found in any of the control animals, however long they live".

More recently, Hesse, Meissner, and Quast (18) and Meissner and Hesse (23) tested an extensive series of complex aniline dyes for their effects on virulent human tubercle bacilli *in vitro*. Those substances showing the highest bactericidal activity were also tested *in vivo*. Of the dyes studied, two azine compounds (safranin and tannin heliotrope) appeared to exert some favourable action on the degree of lung involvement observed in rabbits. The animals in these experiments were first inoculated intratesticularly with virulent human tubercle bacillus, and superinfected four weeks later with bovine organisms injected intravenously.

In view of the chemotherapeutic activity of sulphanilamide and related substances in other infections, a large number of studies have already been published concerning the effects of this group of agents in experimental tuberculosis. Thus, Rich and Follis (24), Greey, Campbell, and Culley (16), Buttle and Parish (3), Ballon and Guernon (1) have reported that after administration of large doses (200 to 300 mgm. daily), sulphanilamide exerted a definite inhibitory effect upon the development of tuberculosis in the guinea pig inoculated subcutaneously with human tubercle bacilli. In particular, there was a striking difference in the macroscopic appearance of the spleens

of the treated and untreated animals. In none of the experiments reported by these investigators, however, was any evidence of curative action demonstrated, and indeed, in many experiments with such doses of sulphanilamide the treated animals died earlier than the controls.

Smithburn (27) on the other hand, concluded that sulphanilamide exerted no beneficial effects in experimental tuberculosis. In his experiments the tubercle bacilli were injected intracerebrally and the drug was administered intra-abdominally. Under such conditions there was no significant difference in the mean survival times of treated and untreated groups of 10 guinea pigs.

Kolmer, Raiziss, and Rule (19) found no demonstrable effect in guinea pigs treated intramuscularly with sulphanilamide, sodium sulphanilamide, and five other azosulphonamide derivatives. The animals were inoculated subcutaneously with virulent human bacilli and treatment with large doses was started two hours after inoculation.

Dietrich (10) also failed to demonstrate any influence of prontosil on the course of tuberculosis in guinea pigs inoculated with the human type of organism, but Birkhaug (2) reports that prontosil soluble (Bayer) exerted a significant action on the development of the infection in guinea pigs inoculated with a bovine strain of tubercle bacilli. Levin (20), however, could detect no effect of the sodium salt of 4-sulphonamidobenzenamidequinoleic acid (Streptal Soluble) upon the course of the infection induced by virulent bovine organisms in guinea pigs.

Corper, Cohn, and Bower (5) have published a good review of the literature on the effects of sulphanilamide in experimental tuberculosis, and on the basis of their observations conclude that sulphanilamide exerts no chemotherapeutic action against the tubercle bacillus in the body, but that the apparently positive findings of Rich and Follis and other workers following administration of massive doses of the drug, are due to toxic effects of sulphanilamide upon the spleen and liver, while the tuberculous process continues. Thus, although the spleens of the treated animals were small, such spleens nevertheless contained as many foci of infection as the enlarged spleens of the untreated controls.

The effects of sulphapyridine and sulphathiazole have been less extensively studied in experimental tuberculosis but, again, the results have been rather variable. Heise and Steenken (17) found that in the disease induced in guinea pigs by inhalation of relatively small doses of virulent human tubercle bacilli, no effect was observed even after prolonged administration of sulphapyridine. A blood concentration of 6.5 to 10 mgm. % was maintained in these experiments over a period of five months of treatment. All the animals were killed six months after inoculation but no difference could be detected in the degree of tuberculosis present in lungs, liver, spleen, and lymph nodes between treated and untreated animals. On the other hand, Feldman and Hinshaw (11) conclude, on the basis of experiments on guinea pigs inoculated subcutaneously with tubercle bacilli, that "sulfapyridine exerted a definite modification and retardation of the expected course and character of the disease

in the animals receiving the drug." The most noticeable effects were in the character and extent of the disease in the spleen and lymph nodes. In these experiments, however, 8 of 20 treated animals died during the first three weeks and the 12 remaining died in from 23 to 56 days. Seven of the control group were killed during this period of time for comparison with seven animals receiving sulphapyridine, and nine of the controls were still alive eight weeks after inoculation. In an addendum to a later paper Feldman, Hinshaw, and Moses (12) state that "the possible effect of sulphathiazole (2 sulphanilamidothiazol) on experimental tuberculosis has also been investigated. The results indicate that this compound has little if any effect on the expected course of the disease".

Climenko (4) reports that the N′-dodecanoylsulphanilamide exerted a highly favourable effect upon the course of experimental tuberculosis in guinea pigs inoculated with human tubercle bacilli. This however has not been confirmed by Steinbach and Duca (28).

Feldman, Hinshaw, and Moses (12, 13, 14) have published a number of papers dealing with the effects of sodium *p*-*p*′-diaminodiphenylsulphone-N-N′-didextrose sulphonate (promin) in experimental tuberculosis, and conclude that, when administered daily to guinea pigs in their food, this substance exerts a notable inhibitory effect on the course of the infection. The animals were inoculated subcutaneously with a dose of 0.0005 mgm. of human tubercle bacilli (Strain *H* 37 *R. V.*), and the amount of drug ingested daily was estimated to be about 300 to 400 mgm. In the control group widespread visceral tuberculosis developed in all cases (11 animals), and the last animal died 189 days after inoculation. At that time 57 out of 68 animals treated with promin (84%) were still living and apparently well. Furthermore, of the 11 treated animals that had died prior to this time none had grossly detectable visceral tuberculosis. At autopsy, the authors state, "only three of the treated animals (4 per cent) had sufficient residual tuberculosis to be detected grossly in the viscera, and in no instance did the disease appear to be sufficiently extensive to cause death of the animal". In connection with these findings, it is of interest to note that the closely related chemical agent diacetyldiaminodiphenylsulphone (rodilone) had previously been reported by Greey, Boddington, and Little (15) to exert no influence upon the course of the disease induced in guinea pigs by subcutaneous inoculation with 0.2 cc. of a suspension of virulent human tubercle containing two to six micro-organisms per oil immersion field. On the other hand, Rist, Block, and Hamon (25) have reported that in rabbits and guinea pigs inoculated intravenously with avian tubercle bacilli both sulphanilamide and diaminodiphenylsulphone exert a definite inhibitory action upon the multiplication of the organisms *in vivo*.

While our own investigation was in progress, Smith, Emmart, and Westfall (26) published a study on the effects of 23 compounds, including certain sulphonamides, sulphones, and related phosphorus compounds, in experimental tuberculosis. In these experiments the effects of the agents were tested both *in vitro* and *in vivo*. Guinea pigs infected with two strains of human

tubercle bacilli and rabbits infected with a bovine strain were employed. It was noted that good inhibition of growth *in vitro* was obtained in decreasing order of magnitude with the following agents:— diaminodiphenylsulphone, diaminodiphenylsulphoxide, sulphathiazole, diaminodiphenylsulphide, sulphadiazine, phosphanilic acid, and promin. Favourable effects *in vivo*, as regards survival time and retardation of the progress of the disease were also observed with diaminodiphenylsulphone, promin, and sulphadiazine. Doubtful effects were obtained with sulphathiazole and bis (dimethylphenyl) phosphinous acid, and irregular results with phosphanilic acid. On the basis of these findings the authors conclude that "the diaminodiphenylsulfone nucleus appears to be a significant point of departure for further investigations in the chemotherapy of tuberculosis".

Procedures

Since there is no valid reason for studying more intensively one class of chemical agents in preference to another, for the purpose of this investigation a number of simple derivatives of aminobenzene were selected for initial study, with the hope that even though the nucleus itself is without action, some lead might be obtained regarding one or other of the substituent groupings, which might point the way for further investigation. In addition, a number of sulphonamide and sulphone derivatives were also tested.

A list of the agents that have been studied is presented below.

List of Compounds Tested

Series I (p-aminobenzene derivatives)

No. 1	*p*-Aminophenol	(*C*)	No. 12	*p*-Aminothiophenol	(*S*)
No. 2	*p*-Aminobenzoic acid	(*C*)	No. 11*a*	Ethyl-*p*-aminophenyl sulphide	(*S*)
No. 3	*p*-Toluidine	(*C*)	No. 44	Ethyl-*p*-aminobenzoate	(*C*)
No. 4	*p*-Ethylaniline	(*C*)	No. 57	2,4-Dichloroaniline	(*C*)
No. 5	*p*-Aminobenzenesulphonic acid (sulphanilic acid)	(*C*)	No. 58	1,2-Dichloroaniline	(*C*)
			No. 61*	N-Diethylamino-isopentyl *p*-aminophenol	(*S*)
No. 6	*p*-Chloroaniline	(*C*)			
No. 7	*p*-Aminophenylacetic acid	(*C*)	No. 69	*p*-Nitroaniline	(*C*)
No. 8	*p*-Phenetidine	(*C*)	No. 70	*p*-Aminobenzonitrile	(*C*)
No. 8*a**	*p*-Aminophenyl hexyl ether	(*S*)	No. 72	*p*-Bromoaniline	(*C*)
No. 10	*p*-Aminophenyl acetate	(*S*)	No. 73	*p*-Iodoaniline	(*C*)
No. 11	*p*-Aminobenzamide	(*S*)	No. 74	*p*-Hexylaminochlorobenzene	(*S*)

Series II (o-aminobenzene derivatives)

No. 12	*o*-Aminophenol	(*C*)	No. 17	*o*-Chloroaniline	(*C*)
No. 13	*o*-Aminobenzoic acid	(*C*)	No. 18	*o*-Aminophenylacetic acid	(*S*)
No. 14	*o*-Toluidine	(*C*)	No. 19	*o*-Phenetidine	(*C*)
No. 15	*o*-Ethylaniline	(*C*)	No. 21	*o*-Aminobenzamide	(*S*)
No. 16	*o*-Aminobenzenesulphonic acid	(*C*)	No. 22	*o*-Aminothiophenol	(*S*)

Series III (m-aminobenzene derivatives)

No. 23	*m*-Aminophenol	(*C*)	No. 29	*m*-Aminophenylacetic acid	(*S*)
No. 24	*m*-Aminobenzoic acid	(*C*)	No. 30	*m*-Phenetidine	(*C*)
No. 25	*m*-Toluidine	(*C*)	No. 31*	*m*-Aminophenyl acetate	(*S*)
No. 26	*m*-Ethylaniline	(*S*)	No. 32	*m*-Aminobenzamide	(*S*)
No. 27	*m*-Aminobenzenesulphonic acid	(*S*)	No. 33	*m*-Aminothiophenol	(*S*)
No. 28	*m*-Chloroaniline	(*C*)			

* *New compounds.*

LIST OF COMPOUNDS TESTED—*Concluded*

Series IV (p-N-ethylaminobenzene derivatives)

No. 34	*p*-N-Ethylaminophenol	(*S*)	No. 39	*p*-N-Ethylaminophenyl ethyl ether	(*S*)
No. 35	*p*-N-Ethylaminobenzoic acid	(*S*)	No. 40*	*p*-N-Ethylaminophenyl acetate	(*S*)
No. 36*	*p*-N-Ethylaminoethylbenzene	(*S*)	No. 41	*p*-N-Ethylaminobenzamide	(*S*)
No. 37	*p*-N-Ethylaminochlorobenzene	(*S*)	No. 45	Ethyl-*p*-N-ethylaminobenzoate	(*S*)

Series V (hydroxychloroanilines)

No. 46	3-Chloro-4-hydroxyaniline	(*S*)	No. 51	2-Hydroxy-5-chloroaniline	(*S*)
No. 47	2-Chloro-4-hydroxyaniline	(*S*)	No. 52	2-Hydroxy-3-chloroaniline	(*S*)
No. 48	2-Hydroxy-4-chloroaniline	(*S*)	No. 53*	2-Hydroxy-6-chloroaniline	(*S*)
No. 49*	3-Hydroxy-4-chloroaniline	(*S*)	No. 54	3-Hydroxy-5-chloroaniline	(*S*)
No. 50*	2-Chloro-5-hydroxyaniline	(*S*)	No. 55	2-Chloro-3-hydroxyaniline	(*S*)

Series VI (diaminodiphenylsulphones)

No. 56	Diaminodiphenylsulphone	(*S*)	Rodilone	(diacetyldiaminodiphenyl-sulphone)	(*2*)
Promin	(sodium *p-p'* diaminodiphenyl-sulphone-N-N'-didextrose sulphonate	(1)			

Series VII (Miscellaneous agents)

No. 59	*o*-Hydroxybenzyl alcohol (saligenin)	(*C*)	No. 68	*p*-Chlorophenol	(*C*)
No. 60	1,2,4-Toluylenediamine	(*C*)	No. 75	Hydroquinone	(*C*)
No. 62	*p*-Nitrophenol	(*C*)	No. 76	Pyrocatechol	(*C*)
No. 63	*p*-Nitrochlorobenzene	(*C*)	No. 77	Resorcinol	(*C*)
No. 64	*p*-Nitrochlorophenol	(*C*)	No. 78	Hydroxyethylapocupreine hydrochloride	(3)
No. 65	β-Naphthylamine	(*C*)		Sulphapyridine and sodium sulpha-pyridine	(*C*)
No. 66	Tetrahydro-β-naphthylamine	(*C*)		Sulphathiazole and sodium sulpha-thiazole	(*C*)
No. 67	Sodium β-naphthoquinone-4-sulphonate	(*C*)			

* *New compounds.*

(C) Commercial preparations, the soluble derivatives of which were made by Dr. Richardson.

(S) Synthesized by Dr. Richardson.

(1) Kindly supplied by Parke, Davis and Co.

(2) Kindly supplied by Laboratory Poulenc Freres of Canada Ltd.

(3) Courtesy of Dr. W. W. G. Maclaclan, Mellon Institute.

Male guinea pigs weighing between 300 and 400 gm. were employed. Most of the animals came from the same source and were usually kept under observation in the laboratory from one to two weeks before being used. The animals were maintained on a diet consisting of oats and hay with liberal amounts of fresh vegetables, either cabbage or turnips.

Each agent was tested (*a*) for toxicity and (*b*) for therapeutic effect, as outlined hereunder.

Toxicity Studies

In order to arrive at some evaluation of toxicity of the various agents the "maximum tolerated dose" and "minimum lethal dose" for the guinea pig were roughly ascertained. This was done by administering increasing doses to each of a series of guinea pigs, and noting the results. A list of the preparations used and the toxicity data obtained with each is given in Table I. Most of the soluble compounds were tested for toxicity both orally and subcutaneously. The insoluble compounds were tested orally only, and were

TABLE I

TOXICITY DATA ON GUINEA PIGS

Compound used	Tested orally, mgm./gm.		Tested subcutaneously, mgm./gm.	
	Max. tol. dose	Lethal dose	Max. tol. dose	Lethal dose
Series I (p-aminobenzene derivatives)				
1 Base (insol.)	2.0	2.5	—	—
1 HCl	2.0	3.0	0.66	1.0
2 HCl	1.0	2.0	0.0625	0.125
3 HCl	1.0	2.0	0.25	0.50
4 HCl	0.25	0.5	0.25	0.50
5 Na salt	5.0	—	5.0	—
6 HCl	0.25	0.50	0.125	0.250
7 HCl	1.0	2.0	1.0	2.0
8 HCl	1.0	2.0	0.25	0.5
8*a* HCl	0.5	1.0	0.125	0.250
9 HCl	1.0	2.0	0.5	0.75
10 HCl	0.5	1.0	0.125	0.25
11 HCl	0.125	0.25	0.125	0.25
11*a* HCl	0.50	1.0	0.25	0.50
44 Insol.	2.0	5.0	—	—
57 Base	0.5	1.0	—	—
58 Base	0.125	0.25	—	—
61 HCl	0.5	1.0	0.25	0.5
69 Base	0.5	1.0	—	—
70 Base	0.25	0.5	—	—
72 HCl	0.25	0.5	0.25	0.5
73 HCl	0.50	1.0	0.25	0.5
74 HCl	0.50	1.0	(Unstable in aqueous solution)	
Series II (o-aminobenzene derivatives)				
12 HCl	1.0	2.0	0.5	1.0
13 HCl	1.0	2.0	0.0625	0.125
14 HCl	0.5	1.0	0.25	0.50
15 HCl	0.5	1.0	0.25	0.50
16 Na salt	3.0	—	1.0	2.0
17 HCl	0.5	1.0	0.25	0.5
18 Na salt	2.0	—	2.0	—
19 HCl	0.5	1.0	0.5	1.0
21 HCl	0.5	1.0	0.5	1.0
22 HCl	0.25	0.5	0.125	0.25
Series III (m-aminobenzene derivatives)				
23 HCl	2.0	3.0	0.125	0.25
24 HCl	1.0	2.0	0.125	0.25
25 HCl	1.0	2.0	0.5	1.0
26 HCl	0.5	1.0	0.25	0.5
27 Na salt	5.0	—	5.0	—
28 HCl	0.5	1.0	0.125	0.25
29 HCl	2.0	—	0.5	1.0
30 HCl	0.5	1.0	0.125	0.25
31 HCl	2.0	3.0	0.125	0.25
32 HCl	2.0	—	0.5	1.0
33 HCl	0.5	1.0	0.25	0.5

TABLE I—*Concluded*

TOXICITY DATA ON GUINEA PIGS—*Concluded*

Compound used	Tested orally, mgm./gm.		Tested subcutaneously, mgm./gm.	
	Max. tol. dose	Lethal dose	Max tol. dose	Lethal dose
Series IV (p-N-ethylaminobenzene derivatives)				
34 Base (insol.)	1.0	2.0	—	—
34 HCl	1.0	2.0	0.25	0.5
35 Na salt	2.0	3.0	3.0	5.0
36 HCl	1.0	2.0	0.125	0.25
37 HCl	0.5	1.0	0.25	0.5
39 HCl	0.5	1.0	0.25	0.5
40 Base (insol.)	2.0	—	—	—
41 Base	1.0	—	—	—
41 HCl	0.5	1.0	0.25	0.5
45 Insol.	2.0	—	—	—
Series V (hydroxychloroanilines)				
45 Base (insol.)	2.0	—	—	—
46 HCl	0.5	1.0	0.5	1.0
47 HCl	1.0	2.0	0.25	0.5
48 HCl	2.0	2.5	0.25	0.5
49 HCl	1.0	2.0	0.25	0.5
50 HCl	0.5	1.0	0.125	0.25
51 HCl	2.0	3.0	0.25	0.5
52 HCl	2.0	3.0	0.125	0.25
53 HCl	2.0	1.0	0.25	0.5
54 HCl	1.0	2.0	0.25	0.5
55 HCl	1.0	2.0	0.125	0.25
Series VI (diaminodiphenylsulphones)				
56 Base	0.5	1.0	—	—
Promin	5.0 (cyanosis)	—	3.0 (cyanosis)	—
Rodilone	2.0	3.0	—	—
Series VII (miscellaneous)				
59 Base	2.0	5.0	—	—
60 Base	2.0	5.0	—	—
62 Base	0.2	0.5	—	—
63 Base	0.5	1.0	—	—
64 Base	0.2	0.5	—	—
65 Base	0.25	0.5	—	—
66 HCl	0.1	0.25	0.05	0.075
67 Na salt	0.5	1.0	0.10	0.20
68 Base	0.5	1.0	—	—
75	0.5	1.0	0.0625	0.125
76	0.125	0.25	0.25	0.5
77	0.5	1.0	0.125	0.25
78	1.0	2.0	0.125	0.25
Sulphapyridine	5.0	—	—	—
Sodium sulphapyridine	—	—	1.0	2.0
Sulphathiazole	5.0	—	—	—
Sodium sulphathiazole	—	—	2.0	4.0

administered in 5% gum acacia suspension by means of a small flexible rubber catheter used as a stomach tube.

Methods of Testing Therapeutic Effects

The therapeutic effects of the soluble agents were tested both after oral and subcutaneous administrations. The insoluble compounds were tested only after oral administration.

The tubercle bacillus (Strain *H* 37 *R.V.*) was obtained from Trudeau, N.Y.,* and cultured on synthetic medium of the following composition: asparagin 5 gm., magnesium citrate 2.5 gm., magnesium sulphate (anhydrous) 0.6 gm., glycerine 20 cc., and distilled water made up to 1000 cc. The pH was adjusted by the addition of sodium hydroxide to 7.4.

The cultures used were generally from two to three weeks old, but younger or older cultures have been used at times, as indicated in the tables. In preparing the inocula, the surface growth was first thoroughly suspended in a small volume of 0.9% sterile saline to form a thick uniform suspension. A measured small volume of this was then evaporated to dryness on a water-bath and the residue weighed. From this by subtracting the weight of the sodium chloride the "dried weight" of organisms per cubic centimetre of suspension was calculated. Another portion of the original suspension was then suitably diluted with saline so that the inoculating dose being employed was contained in 0.25 cc. This dose varied from 0.25 to 1 mgm. in most experiments, but in a few cases smaller quantities of from 0.1 to 0.001 mgm. 'dried weight' were employed.

The organisms were injected intraperitoneally in all experiments.

In most experiments a large group of 40 to 50 guinea pigs were employed at one time. These were divided into several small groups, one of which was kept as untreated controls, while the other groups were used for testing different agents. Each animal in such a series received the same dose of the same suspension. This procedure permits a comparison of treated groups not only with the untreated control animals, but also with one another, which is a definite advantage in view of the variability of the tuberculous process in the guinea pig, as judged by duration of survival after inoculation.

In order to bring out even the slightest effect of the agent being tested, in all cases the initial dose of the substance was administered from two to three hours prior to inoculation in the oral tests and 30 min. prior to inoculation in the subcutaneous tests, subsequent treatments being given daily or more frequently thereafter as indicated in the tables.

It should be emphasized that the aim of treatment in these studies was to submit the tubercle bacillus *in vivo* to the maximum possible concentration of the chemical agent as early as possible after inoculation. The doses employed were therefore as high as could be tolerated. Under such conditions, it was not considered important to maintain treatment for very long periods of time,

* *Courtesy of Dr. W. Steenken Jr.*

and in some experiments owing to the development of early toxic effects (marked loss of weight particularly) treatment had to be discontinued as early as three to five days after inoculation. In most experiments treatment was continued from 7 to 10 days, and in a few as long as 15 to 28 days.

The animals were weighed daily during the treatment period, and weekly thereafter until death; if an animal survived the untreated controls it was usually observed until death or killed in from six to seven months after inoculation.

All animals were autopsied, and the spleen, liver, kidneys, and lymph glands examined for gross evidence of tuberculosis. It was not considered necessary to make microscopic examinations of the tissues of the animals as gross evidence of tuberculosis was detected in varying degrees in all animals, even in those surviving inoculation for six to seven months.

Results

The results obtained with the different series of agents listed above are summarized in Tables II and VIII.

TABLE II

THERAPEUTIC DATA ON GUINEA PIGS

Series I (*p*-aminobenzene derivatives)

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 1 Base Oral	0.5	5	0.25	14	14, 16, 16, 29, 33, 35, 39, 40, 48	30.0	14, 16, 16, 29, 35, 37, 40, 43, 49	30.7
	1.0	3	0.5	17	33, 38, 85	52.0	31, 33, 36, 36, 40, 167	57.1
No. 1 HCl Subcutaneous	0.25	4	1.0	17	43, 148+	95.5	30, 45, 48, 72	48.7
	0.25	4	0.5	15	20, 28, 33, 38	29.7	35, 35, 35, 38	35.7
	0.30	5	0.25	14	25, 27, 29, 37, 39, 89+	39.3	14, 16, 16, 29, 35, 37, 40, 43, 49	30.7
	0.30	5	0.2	14	22, 22, 35, 35, 39, 42, 44, 56, 63	39.8	24, 39, 40, 42, 42, 42, 44, 47, 70, 120	51.3
	0.30	4	0.1	21	29, 35, 38, 43, 44	37.8	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.30	9	0.01	36	21, 38, 68	42.3	47, 65, 84, 161	89.2
	0.30	10	0.01	30	78, 183, 199, 203, 205+, *K*		61, 75, 126, 144, 203	121.8
	0.30	10	0.001	18	84, 123+, 128+, 148+, 180+, *K*		92, 120, 122, 122	113.5
	0.30	6	0.001	17	50, 56, 61, 91	64.5	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
	0.35	4	1.0	17	20, 49+	34.5	17, 19, 21, 25	20.5
	0.40	5	0.5	14	29, 58, 112+, 121+	80.0	31, 39, 39, 43, 60	42.4

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE II—*Continued*

THERAPEUTIC DATA ON GUINEA PIGS—*Continued*

Series I (*p*-aminobenzene derivatives)—*Continued*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 2 HCl Oral	0.25	4	0.5	14	29	29.0	28, 28, 29, 36, 131	50.4
	0.125	8	0.5	14	23, 28	25.5	24, 28, 28, 36	29.0
No. 2 HCl Subcutaneous	0.05	8	0.5	14	22, 23	22.5	24, 28, 28, 36	29.0
	0.0625	4	1.0	15	14, 21	17.5	25, 28, 58, 99	52.5
No. 3 HCl Oral	0.25	4	1.0	17	21	21.0	17, 19, 21, 25	20.5
	0.15	4	0.5	14	28, 155+	91.5	28, 28, 29, 36, 131	50.4
No. 3 HCl Subcutaneous	0.13	3	1.0	14	50	50.0	17, 19, 21, 25	20.5
	0.10	4	0.5	14	28, 35, 123	57.3	28, 28, 29, 36, 131	50.4
No. 4 HCl Oral	0.075	4	0.25	14	28, 35, 50+, 88+, 106+	61.4	14, 16, 16, 29, 35, 37, 40, 43, 49	30.7
	0.075	5	0.20	14	39, 42, 59	46.6	24, 39, 40, 42, 42, 42, 44, 47, 70, 120	51.3
	0.075	10	0.10	21	35, 37, 54+, 84+	52.5	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.075	10	0.01	36	112, 163+	137.5	47, 65, 84, 161	89.2
	0.075	10	0.001	18	98, 110, 147+, 170+	131.3	92, 120, 122, 122	113.5
	0.075	15	0.001	17	56, 57, 119, 129, 149	102.0	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
	0.13	3	1.0	17	14, 180+	97.0	17, 19, 21, 25	20.5
No. 4 HCl Subcutaneous	0.02	3	1.0	15	21, 32+	26.5	17, 19, 21, 25	20.5
No. 5 Na salt Oral	2.0	4	1.0	15	28, 42	35.0	45, 49, 52, 138	71.0
No. 5 Na salt Subcutaneous	0.75	5	0.5	15	27, 35	31.0	35, 35, 35, 38	35.7
	1.25	4	1.0	15	22, 30	26.0	25, 28, 58, 99	52.5
No. 6 Base Oral	0.1	12	0.25	18	30, 31, 32, 38, 52, 73, 83	43.1	28, 31, 31, 32, 47, 49, 70, 95, 108, 180	67.1
	0.125	10	0.25	22	55, 66, 129+	83.3	28, 58, 71, 76, 125	71.6
	0.125	12	0.25	15	50, 55, 63, 65, 142, 165+, 180+, 186+	113.2	31, 36, 40, 49, 50, 109, 124, 142, 162	87.9
No. 6 HCl Oral	0.05	8	0.25	21	26, 27, 29, 37	29.8	21, 27, 28, 30, 38	28.8
	0.05	8	0.05	21	17, 18, 24, 47+	26.5	14, 18, 23, 26, 27	21.6
	0.0625	4	1.0	15	45, 180+, *K*		45, 49, 52, 138	71.0
	0.075	5	0.5	14	28, 85+, 126+	79.7	33, 33, 63, 66	48.7
	0.075	10	0.25	10	16, 17, 19, 21, 24, 39+, 63+, 64+, 71+	37.9	18, 19, 21, 21, 21, 23, 25, 26, 26, 27	22.7
	0.075	7	0.5	14	19, 20, 20, 22, 22, 22, 28+, 50+, 51+, 122+	37.8	21, 25, 26	24.0

NOTE: + = *Survived longer than all the untreated controls of the same series.*

K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE II—*Continued*

THERAPEUTIC DATA ON GUINEA PIGS—*Continued*

Series I (*p*-aminobenzene derivatives)—*Continued*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 6 HCl	0.075	12	0.25	18	30, 30, 31, 31, 31, 52, 52, 55, 59, 82	45.3	28, 31, 31, 32, 47, 49, 70, 95, 108, 180	67.1
	0.075	5	0.01	36	56, 64, 73, 91	71.0	47, 65, 84, 161	89.2
	0.075	10	0.01	30	78, 87, 112, 140, 155	114.4	61, 75, 126, 144, 203	121.8
	0.075	10	0.001	18	157, 176+, *K*, 176+, *K*, 176+, *K*, 176+, *K*		92, 120, 122, 122	113.5
	0.075	15	0.001	17	71, 85, 170 *K*		41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
	0.10	10	0.25	22	28, 28, 84, 133+, 204+, K		28, 58, 71, 76, 125	71.6
	0.10	12	0.25	15	36, 51, 51, 57, 62, 80, 124, 156, 180+, *K*		31, 36, 40, 49, 50, 109, 124, 142, 162	87.9
	0.10	12	0.25	13	19, 19, 19, 20, 20, 20, 24, 26, 26, 29, 31, 33, 33, 33, 40, 41, 70, 103	43.7	16, 17, 19, 24, 26, 26, 26, 26, 27, 27, 27, 27, 27, 28, 30, 30, 33, 33, 35, 180 *K*, 180 *K*, 180 *K*	
No. 6 HCl Subcutaneous	0.025	5	0.25	21	24, 26, 28, 29	27.0	21, 27, 28, 30, 38	28.8
	0.025	8	0.5	21	19, 21, 22, 24, 31+	23.4	14, 18, 23, 26, 27	21.6
	0.05	5	0.5	14	35, 82+, 93+	70.0	33, 33, 63, 66	48.7
	0.05	6	0.25	10	19, 19, 19, 21, 21, 23, 28+, 58+, 69+	30.8	18, 19, 21, 21, 21, 23, 25, 26, 26, 27	22.7
	0.05	4	0.5	14	17, 19, 22, 22, 23, 24, 27+, 32+, 84+	29.3	21, 25, 26	24.0
	0.05	10	0.001	18	81, 101, 170 *K*, 170 *K*, 170 *K*		92, 120, 122, 122	113.5
	0.05	15	0.001	17	54, 66, 75, 79, 131	61.0	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 K	
	0.075	10	0.25	22	63, 107, 121	93.3	28, 58, 71, 76, 125	71.6
	0.075	12	0.25	13	16, 16, 16, 19, 19, 19, 19, 19, 20, 20, 21, 21, 24, 26, 26, 28, 33, 64, 72	26.2	16, 17, 19, 24, 26, 26, 26, 26, 27, 27, 27, 27, 27, 28, 30, 30, 33, 33, 35, 180 *K*, 180 *K*, 180 *K*	
	0.075	4	0.50	14	180+, *K*		28, 28, 29, 36, 131	50.4
No. 7 HCl Oral	0.25	4	1.0	15	35, 180+	107.5	45, 49, 52, 138	71.0
	0.25	8	0.5	14	30, 35	32.5	24, 28, 28, 36	29.0
No. 7 HCl Subcutaneous	0.25	4	1.0	15	23, 28	25.5	25, 28, 58, 99	52.5
No. 8 HCl Oral	0.25	4	1.0	15	21	21.0	88	88.0

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE II—*Continued*

THERAPEUTIC DATA ON GUINEA PIGS—*Continued*

Series I (*p*-aminobenzene derivatives)—*Continued*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 8 HCl Subcutaneous	0.0625	4	1.0	17	46, 48	47.0	30, 45, 48, 72	48.7
	0.075	5	0.5	14	26, 33, 35, 36, 142+	55.0	31, 39, 39, 43, 60	42.4
	0.125	4	1.0	15	28, 29	28.5	25, 28, 58, 99	52.5
No. 8α HCl Oral	0.10	6	0.5	15	47+, 121+	84.0	36, 36, 36, 42	37.5
	0.10	5	0.2	14	47, 99	73.0	24, 39, 40, 42, 42, 42, 44, 47, 70, 120	51.3
	0.10	10	0.10	21	33, 36, 39, 45, 49+	40.4	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.10	10	0.01	10	72, 105, 108, 133	104.5	47, 65, 84, 161	89.2
	0.10	10	0.001	18	127, 141+, 157+, 158+, 176+	151.8	92, 120, 122, 122	113.5
	0.10	15	0.001	17	50, 85, 105, 162	100.5	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
No. 8α HCl Subcutaneous	0.025	4	0.5	15	18	18.0	21, 25, 26	24.0
	0.05	4	0.2	14	118	118.0	24, 39, 40, 42, 42, 42, 44, 47, 70, 120	51.3
No. 9 HCl Oral	0.02	4	0.5	14	35, 56	45.5	28, 28, 29, 36, 131	50.4
	1.0	3	1.0	15	22, 39	30.5	25, 25, 25, 27, 42, 42	31.0
No. 9 HCl Subcutaneous	0.125	4	1.0	15	22, 28	25.0	25, 28, 58, 99	52.5
No. 10 HCl Oral	0.125	4	1.0	15	36, 51	43.5	45, 49, 52, 138	71.0
No. 10 HCl Subcutaneous	0.05	4	1.0	15	24, 85	54.5	25, 28, 58, 99	52.5
	0.10	5	0.2	14	72, 70	71.0	24, 39, 40, 42, 42, 42, 44, 47, 70, 120	51.3
No. 11 HCl Oral	0.04	4	1.0	15	40, 44	42.0	45, 49, 52, 138	71.0
No. 11 HCl Subcutaneous	0.0158	4	1.0	17	28, 41	34.5	30, 45, 48, 72	48.7
	0.05	3	0.5	15	28	28.0	28, 28, 29, 36, 131	50.4
No. 44 Ester Oral	0.5	6	0.5	16	40, 147+	93.5	36, 36, 36, 42	37.5
	1.0	6	0.25	21	87, 180+, *K*		31, 33, 33, 149	62.0
	1.0	5	0.25	26	41, 49, 58, 219+, K		35, 39, 42, 49, 207	74.4
	1.0	5	0.1	28	28, 58, 91, 191+	92.0	22, 26, 35, 37, 40, 40, 43, 47, 175	51.3
	1.0	3	0.1	21	33, 38, 45, 46	40.5	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	1.0	10	0.01	30	50, 56, 63, 87, 182	87.6	61, 75, 126, 144, 203	121.8
	1.0	5	0.001	28	112, 154, 176+	114.0	63, 71, 71, 74, 84, 100, 100, 133, 140, 162	99.6
	1.0	10	0.001	18	86, 124+, 124+, 127+	115.2	92, 120, 122, 122	113.5
	2.0	2	0.25	18	27, 137+	82.0	20, 32, 34, 41	31.8

NOTE: + = *Survived longer than all the untreated controls of the same series.*

K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE II—*Concluded*

THERAPEUTIC DATA ON GUINEA PIGS—*Concluded*

Series I (*p*-aminobenzene derivatives)—*Concluded*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 57 Base Oral	0.125	10	0.1	21	29, 39	34.0	26, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.125	10	0.01	30	75, 112, 204+, *K*, 204+, *K*		61, 75, 126, 144, 203	121.8
	0.125	10	0.001	31	67, 127, 212+, *K*, 212+, *K*, 212+, *K*		106, 126, 141, 203, 210	157.2
	0.125	15	0.001	17	39, 79, 170 *K*, 170 *K*, 170 *K*		41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
No. 58 Base Oral	0.4	10	0.001	10	45, 130 *K*		46, 130 *K*, 130 *K*, 130 *K*	
No. 61 HCl Oral	0.125	6	0.1	13	40, 86	63.0	29, 64, 105, 114	78.0
No. 61 HCl Subcutaneous	0.075	6	0.1	13	58	58.0	29, 64, 105, 114	78.0
No. 69 Base Oral	0.125	6	0.1	13	36, 133+	84.5	29, 64, 105, 114	78.0
	0.125	10	0.001	10	82, 82	82.0	46, 130 *K*, 130 *K*, 130 *K*	
No. 70 Base Oral	0.0625	10	0.001	10	45, 46	45.5	46, 130 *K*, 130 *K*, 130 *K*	
No. 72 HCl Oral	0.05	9	0.1	33	127, 180 *K*		72, 133, 180 *K*, 180 *K*	
	0.05	10	0.001	10	46, 130 *K*		46, 130 *K*, 130 *K*, 130 *K*	
No. 72 HCl Subcutaneous	0.05	9	0.1	33	129, 180 *K*		72, 133, 180 *K*, 180 *K*	
	0.05	10	0.001	10	38, 45	41.5	46, 130 *K*, 130 *K*, 130 *K*	
No. 73 HCl Oral	0.1	10	0.1	33	127, 168	147.0	72, 133, 180 *K*, 180 *K*	
	0.1	10	0.001	10	46, 124	85.0	46, 130 *K*, 130 *K*, 130 *K*	
No. 73 HCl Subcutaneous	0.05	10	0.1	33	38, 162	100.0	72, 133, 180 *K*, 180 *K*	
	0.05	10	0.001	10	46, 130 *K*		46, 130 *K*, 130 *K*, 130 *K*	
No. 74 HCl Oral	0.125	10	0.1	33	80, 162	121.0	72, 133, 180 *K*, 180 *K*	
	0.125	10	0.001	10	46, 130 *K*		46, 130 *K*, 130 *K*, 130 *K*	

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE III

THERAPEUTIC DATA ON GUINEA PIGS

Series 2 (*o*-aminobenzene derivatives)

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average days
No. 12 HCl Oral	0.25	4	1.0	15	37, 47	42.0	45, 49, 52, 138	71.0
No. 12 HCl Subcutaneous	0.125	4	1.0	17	29, 35	32.0	30, 45, 48, 72	48.7
No. 13 HCl Oral	0.25	4	1.0	15	30, 36	33.0	45, 49, 52, 138	71.0
No. 13 HCl	0.0625	4	1.0	15	25, 25, 27, 36	28.2	25, 25, 25, 27, 42, 42	31.0
Subcutaneous	0.075	4	0.5	14	26, 98+	62.0	31, 39, 39, 43, 60	42.4
No. 14 HCl	0.038	5	0.5	15	28, 30	29.0	35, 35, 35, 38	35.7
Oral	0.05	8	0.5	15	23, 36	28.5	24, 28, 28, 36	29.0
No. 14 HCl Subcutaneous	0.0625	4	1.0	17	28, 28	28.0	30, 45, 48, 72	48.7
No. 15 HCl	0.10	4	0.5	14	35	35.0	28, 28, 29, 36, 131	50.4
Oral	0.125	4	1.0	15	28	28.0	45, 49, 52, 138	71.0
No. 15 HCl	0.05	4	0.5	14	28, 45	36.5	28, 28, 29, 36, 131	50.4
Subcutaneous	0.0625	4	1.0	15	28	28.0	25, 28, 58, 99	52.5
No. 16 Na salt Oral	0.25	4	1.0	15	38, 46	42.0	25, 25, 25, 27, 42, 42	31.0
No. 16 Na salt Subcutaneous	0.25	4	0.5	15	35, 180+	107.5	31, 33, 36, 36, 40, 167	57.1
No. 17 HCl Oral	0.125	4	1.0	15	35, 41	38.0	45, 49, 52, 138	71.0
No. 17 HCl	0.05	3	0.5	15	27, 31	29.0	35, 35, 35, 38	35.7
Subcutaneous	0.0625	4	1.0	17	26, 28	27.0	30, 45, 48, 72	48.7
No. 18 Na salt Oral	1.0	3	0.5	15	32, 105	68.5	31, 33, 36, 36, 40, 167	57.1
No. 18 Na salt Subcutaneous	0.5	3	0.5	15	26, 33	34.5	31, 33, 36, 36, 40, 167	57.1
No. 19 HCl Oral	0.0625	4	1.0	15	12, 14	13.0	88	88.0
No. 19 HCl	0.0315	4	1.0	17	72, 72	72.0	30, 45, 48, 72	48.7
Subcutaneous	0.05	4	0.5	14	26, 38, 42, 64+	42.5	31, 39, 39, 43, 60	42.4
No. 21 HCl Oral	0.1	4	0.5	15	26, 91	58.5	28, 28, 29, 36, 131	50.4

NOTE: + = *Survived longer than all the untreated controls of the same series.*

TABLE III—*Concluded*

THERAPEUTIC DATA ON GUINEA PIGS—*Concluded*

Series 2 (*o*-aminobenzene derivatives)—*Concluded*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 21 HCl Subcutaneous	0.125	4	1.0	15	20, 42	31.0	25, 28, 58, 99	52.5
No. 22 HCl	0.04	5	0.5	15	24, 33	28.5	35, 35, 35, 38	35.7
Oral	0.0625	4	1.0	15	35, 41	38.0	45, 49, 52, 138	71.0
No. 22 HCl	0.025	4	0.5	15	21, 23, 42, 43	32.2	28, 28, 29, 36, 131	50.4
Subcutaneous	0.035	4	1.0	15	22, 23	22.5	25, 28, 58, 99	52.5

TABLE IV

THERAPEUTIC DATA ON GUINEA PIGS

Series 3 (*m*-aminobenzene derivatives)

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 23 HCl Oral	0.5	4	1.0	15	28, 37	32.5	45, 49, 52, 138	71.0
No. 23 HCl Subcutaneous	0.035	4	1.0	15	25, 28	26.5	25, 28, 58, 99	52.5
No. 24 HCl	0.125	5	0.5	15	31, 39	35.0	35, 35, 35, 38	35.7
Oral	0.125	4	1.0	15	28, 35	31.5	45, 49, 52, 138	71.0
No. 24 HCl	0.025	5	0.5	15	28, 32	30.0	35, 35, 35, 38	35.7
Subcutaneous	0.03125	4	1.0	15	28, 28	28.0	30, 45, 48, 72	48.7
No. 25 HCl Oral	0.25	4	1.0	15	46, 46	46.0	45, 49, 52, 138	71.0
No. 25 HCl Subcutaneous	0.0625	4	1.0	17	25, 38	31.5	30, 45, 48, 72	48.7
No. 26 HCl	0.125	4	1.0	15	32, 33	32.5	88	88.0
Oral	0.125	5	0.5	15	10, 21	15.5	35, 35, 35, 38	35.7

TABLE IV—*Concluded*

THERAPEUTIC DATA ON GUINEA PIGS—*Concluded*

Series 3 (*m*-aminobenzene derivatives)—*Concluded*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 26 HCl Subcutaneous	0.125	4	0.5	14	35, 36	35.5	28, 28, 29, 36, 131	50.4
No. 27 Na salt Oral	1.0	3	1.0	15	16, 27	21.5	25, 25, 25, 27, 42, 42	31.0
	2.0	3	0.5	14	36	36.0	31, 33, 36, 36, 40, 167	57.1
No. 27 Na salt Subcutaneous	1.25	4	0.5	14	34, 91	62.5	31, 33, 36, 36, 40, 167	57.1
No. 28 HCl Oral	0.1	4	0.5	14	33, 33	33.0	28, 28, 29, 36, 131	50.4
No. 28 HCl Subcutaneous	0.04	4	0.5	14	25, 30, 36, 44	34.5	28, 28, 29, 36, 131	50.4
	0.05	4	1.0	17	18, 24	21.0	21, 28	24.5
No. 29 HCl Oral	0.5	4	1.0	15	47, 54	50.5	88	88.0
No. 29 HCl Subcutaneous	0.125	4	1.0	15	41, 99+	70.0	88	88.0
No. 30 HCl Oral	0.1	3	0.5	15	26, 35	30.5	28, 28, 29, 36, 131	50.4
No. 30 HCl Subcutaneous	0.0625	4	1.0	17	21, 28	24.5	18, 24	21.0
No. 31 HCl Oral	0.5	4	1.0	15	33, 46	34.5	88	88.0
No. 31 HCl Subcutaneous	0.04	4	1.0	15	67, 180+	123.5	88	88.0
	0.04	4	0.5	14	24, 24	24.0	21, 25, 36	24.0
No. 32 HCl Oral	0.5	4	1.0	15	40, 50	45.0	88	88.0
No. 32 HCl Subcutaneous	0.075	4	0.5	15	30, 40	35.0	28, 28, 29, 36, 131	50.4
	0.125	4	1.0	15	39	39.0	39, 40, 51	43.3
No. 33 HCl Oral	0.125	4	1.0	15	42, 42	42.0	39, 40, 51	43.3
No. 33 HCl	0.0625	5	0.2	14	44, 55	49.5	24, 39, 40, 42, 42, 42, 44, 47, 70, 120	51.3

NOTE: + = *Survived longer than all the untreated controls of the same series.*

TABLE V

THERAPEUTIC DATA ON GUINEA PIGS

Series 4 (*p*-N-ethylaminobenzene derivatives)

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 34 HCl Oral	0.5	6	0.5	16	51+	51.0	36, 36, 36, 42	37.5
	0.5	10	0.001	31	114, 126, 128, 187, 189	148.8	106, 126, 141, 203, 210	157.2
No. 34 HCl Subcutaneous	0.075	6	0.5	16	52+, 107+	79.5	36, 36, 36, 42	37.5
	0.075	6	0.25	11	26, 30	28.0	21, 23, 38, 120	50.4
	0.1	10	0.1	21	28, 33, 33, 40, 49+	36.6	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.1	10	0.001	31	142, 212+, *K*, 212+, *K*, 212+, *K*		106, 126, 141, 203, 210	157.2
No. 35 Na salt Oral	0.5	5	1.0	12	85, 205+	145.0	63, 65, 84, 118	82.5
No. 35 Na salt Subcutaneous	0.5	5	1.0	5	51, 56	53.5	63, 65, 84, 118	82.5
No. 36 HCl Oral	0.25	5	1.0	12	64, 66	65.0	63, 65, 84, 118	82.5
No. 36 HCl Subcutaneous	0.03	5	1.0	12	14, 85	54.5	63, 65, 84, 118	82.5
No. 37 HCl Oral	0.02	6	0.25	21	31, 32, 33, 41	34.2	31, 33, 33, 149	62.0
No. 37 HCl Subcutaneous	0.0125	6	0.25	21	19, 28, 30, 34	27.7	31, 33, 33, 149	62.0
	0.0125	5	0.001	31	68, 126, 206	133.3	106, 126, 141, 203, 210	157.2
No. 39 HCl Oral	0.125	4	1.0	15	42, 48	45.0	39, 40, 51	43.3
No. 39 HCl Subcutaneous	0.075	4	1.0	15	20, 42	31.0	39, 40, 51	43.3
No. 40 Base Oral	1.0	5	0.25	14	24, 26	25.0	25, 28, 28, 28, 28, 31	28.0
	1.0	7	0.10	21	28, 31	24.5	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
No. 41 HCl Oral	0.1	6	0.25	21	28, 41	34.5	31, 33, 33, 149	62.0
No. 41 HCl Subcutaneous	0.075	6	0.25	21	22, 30	26.0	31, 33, 33, 149	62.0
No. 45 Base (Insoluble) Oral	0.5	6	0.5	16	38	38.0	36, 36, 36, 42	37.5
	0.5	6	0.5	21	36	36.0	31, 33, 33, 149	62.0

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series,—no average given.*

TABLE VI

THERAPEUTIC DATA ON GUINEA PIGS

Series 5 (Hydroxychloroanilines)

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm /gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 46 HCl Oral	0.25	6	0.5	16	35, 41	38.0	36, 36, 36, 42	37.5
	0.50	5	0.25	26	32, 50, 105, 217+	101.0	35, 39, 42, 49, 207	74.4
No. 46 HCl Subcutaneous	0.125	6	0.25	11	154+, 180+	157.0	21, 23, 38, 120	50.4
	0.125	5	0.25	26	40, 40, 41	40.3	35, 39, 42, 49, 207	74.4
No. 47 HCl Oral	0.25	6	0.25	11	30, 127+	78.5	21, 23, 38, 120	50.4
	0.25	9	0.01	30	75, 117, 121, 126, 147	117.2	61, 75, 126, 144, 203	121.8
	0.50	10	0.001	31	120, 159, 193, 212+, *K*		106, 126, 141, 203, 210	157.2
	0.50	10	0.001	10	56, 120 *K*		46, 130 *K*, 130 *K*, 130 *K*	
No. 47 HCl Subcutaneous	0.1	6	0.25	11	26, 154	90.0	21, 23, 38, 120	50.4
	0.1	9	0.01	30	33, 56, 103, 141, 178	102.2	61, 75, 126, 144, 203	121.8
	0.1	10	0.001	31	84, 108, 212+, *K*, 212+, *K*, 212+, *K*		106, 126, 141, 203, 210	157.2
	0.1	10	0.001	10	77, 86, 130 *K*		46, 130 *K*, 130 *K*, 130 K	
No. 48 HCl Oral	0.5	6	0.25	21	33, 34	33.5	31, 33, 33, 149	62.0
No. 48 HCl Subcutaneous	0.1	6	0.25	21	21, 88	54.5	31, 33, 33, 149	62.0
No. 49 HCl Oral	0.50	6	0.25	21	21, 99	60.0	31, 33, 33, 149	62.0
No. 49 HCl Subcutaneous	0.125	6	0.25	21	21, 24	22.5	31, 33, 33, 149	62.0
No. 50 HCl Oral	0.1	5	0.1	28	37, 38, 40, 42, 54	42.4	22, 26, 35, 37, 40, 40, 43, 44, 175	51.3
	0.1	6	0.1	21	35, 36, 49+, 49+	42.2	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.125	6	0.25	11	180+, *K*, 180+, *K*		21, 23, 38, 120	50.4
	0.125	7	0.5	18	20, 21, 21, 24, 25, 25, 25, 33, 112, 192	49.8	18, 20, 20, 23, 23, 23, 24, 32, 38, 108, 161, 204 *K*	
	0.125	5	0.25	14	22, 22, 29, 121+	48.5	25, 28, 28, 28, 28, 31	28.0
	0.15	4	0.25	18	28, 32, 34, 180+, *K*		20, 32, 34, 41	31.8
	0.10	6	0.01	36	22, 24, 39, 63, 80	45.6	47, 65, 84, 161	89.2
	0.10	5	0.001	28	81, 95, 96, 126, 191+, *K*		63, 71, 71, 74, 84, 100, 100, 133, 140, 162	99.6

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE VI—*Continued*

THERAPEUTIC DATA ON GUINEA PIGS—*Continued*

Series 5 (Hydroxychloroanilines)—*Continued*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 50 HCl Oral	0.10	10	0.001	18	120, 168+, *K*, 168+, *K*, 168+, *K*, 168+, *K*		92, 120, 122, 122	113.5
No. 50 HCl Subcutaneous	0.05	7	0.5	18	18, 35, 54, 56, 88, 94, 94, 116, 117, 140	81.2	18, 20, 20, 23, 23, 23, 24, 32, 38, 108, 161, 204 *K*	
	0.05	5	0.25	17	14, 14, 14, 35, 35, 54, 112, 116, 142, 162+, 180+, *K*		14, 14, 21, 23, 28, 31, 33, 35, 39, 56, 107, 151	46.7
	0.05	5	0.1	28	35, 35, 36, 181+	71.7	22, 26, 35, 37, 40, 40, 43, 44, 175	51.3
	0.05	6	0.1	21	18, 21, 25, 35	24.7	21, 28, 29, 32, 35, 35, 35, 36, 46	32.9
	0.05	5	0.001	28	50, 50, 53, 70, 98	64.2	63, 71, 71, 74, 84, 100, 100, 133, 140, 162	99.6
	0.0625	6	0.25	11	29, 87	58.0	21, 23, 38, 120	50.4
	0.075	4	0.25	18	57+, 103+	80.0	20, 32, 34, 41	31.8
	0.05	10	0.01	30	42, 45, 46, 63, 205+, *K*		61, 75, 126, 144, 203	121.8
	0.05	10	0.001	18	114, 121, 129+, 169+, *K*, 169+, *K*		92, 120, 122, 122	113.5
No. 51 HCl Oral	0.5	6	0.25	11	24, 30	27.0	21, 23, 28, 120	50.4
No. 51 HCl Subcutaneous	0.125	6	0.25	11	32, 35	33.5	21, 23, 28, 120	50.4
No. 52 HCl Oral	0.5	6	0.5	18	109, 126	117.5	18, 20, 20, 23, 23, 23, 24, 32, 108, 161, 204 *K*	
	0.5	4	0.25	14	24, 24, 26, 219+, *K*		25, 28, 28, 28, 28, 31	28.0
	0.75	5	0.25	26	41, 120, 126	95.6	35, 39, 42, 49, 207	74.4
	0.50	10	0.01	30	98, 122, 130, 143, 173	133.3	61, 75, 126, 144, 203	121.8
No. 52 HCl Subcutaneous	0.05	6	0.5	18	23, 77	50.0	18, 20, 20, 23, 23, 23, 24, 32, 108, 161, 204 *K*	
	0.075	5	0.25	26	38, 44, 45, 52	44.7	35, 39, 42, 49, 207	74.4
	0.10	5	0.25	14	20, 22, 23, 54+, 57+	35.2	25, 28, 28, 28, 28, 31	28.0
No. 53 HCl Oral	0.25	6	0.5	18	35, 180	107.5	18, 20, 20, 23, 23, 23, 24, 32, 108, 161, 204 *K*	
	0.25	5	0.25	26	53	53.0	35, 39, 42, 49, 207	74.4
	0.25	10	0.01	30	91, 147, 162, 205+, *K*, 205+, *K*		61, 75, 126, 144, 203	121.8

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE VI—*Concluded*

THERAPEUTIC DATA ON GUINEA PIGS—*Concluded*

Series 5 (Hydroxychloroanilines)—*Concluded*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 53 HCl Subcutaneous	0.05	6	0.5	18	15, 129	72.0	18, 20, 20, 23, 23, 23, 24, 32, 108, 161, 204 *K*	
	0.075	5	0.25	26	36, 43	38.5	35, 39, 42, 49, 207	74.4
No. 54 HCl Oral	0.5	6	0.25	17	53, 79	66.0	14, 14, 21, 23, 28, 31, 33, 35, 39, 56, 107, 151	46.7
	0.5	5	0.25	26	50, 61, 186	65.6	35, 39, 42, 49, 207	74.4
	0.5	10	0.01	30	106, 108, 112, 122, 157	121.0	61, 75, 126, 144, 203	121.8
No. 54 HCl Subcutaneous	0.05	5	0.25	26	40, 47, 50, 60	49.2	35, 39, 42, 49, 207	74.4
No. 55 HCl Oral	0.5	5	0.25	26	43, 63	53.0	35, 39, 42, 49, 207	74.4
No. 55 HCl Subcutaneous	0.0625	5	0.25	26	53, 61	57.0	35, 39, 42, 49, 207	74.4

NOTE: K = *Killed to complete series,—no average given.*

TABLE VII

THERAPEUTIC DATA ON GUINEA PIGS

Series 6 (Diaminodiphenylsulphones)

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 56 (Base) Oral	0.125	5	0.25	14	23, 26	24.5	25, 28, 28, 28, 31	28.0
	0.125	10	0.10	21	42, 43	42.5	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.125	10	0.10	36	77, 82, 89	82.7	47, 65, 84, 161	89.2
	0.125	5	0.001	31	85, 100, 127, 141	113.2	106, 126, 141, 203, 210	157.2
	0.125	7	0.001	17	75, 170 *K*		41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	

NOTE: *K* = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE VII—*Concluded*

THERAPEUTIC DATA ON GUINEA PIGS—*Concluded*

Series 6 (Diaminodiphenylsulphones)—*Concluded*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
Promin (salt)	1.0	6	0.25	17	39, 39, 42, 45, 46, 112	53.8	14, 14, 21, 23, 28, 31, 33, 35, 39, 56, 107, 151	46.7
Oral	0.5	5	0.25	14	22, 23, 25, 29, 31, 96+	37.7	25, 28, 28, 28, 28, 31	28.0
	0.5	5 (2× daily)	0.10	28	23, 23, 25, 35, 45, 49, 50, 70, 141	51.2	22, 26, 35, 37, 40, 40, 43, 44, 175	51.3
	0.5	5	0.01	36	55, 85, 103	81.0	47, 65, 84, 161	89.2
	0.5	(2× daily) 10	0.01	30	86, 98, 150, 168, 205+	141.4	61, 75, 126, 144, 203	121.8
	0.5	5 (2× daily)	0.001	28	70, 98, 100, 106, 133, 137, 142	112.3	63, 71, 71, 74, 84, 100, 100, 133, 140, 162	99.6
	0.5	15	0.001	17	49, 56, 78, 87, 113	76.6	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
	0.5	28	0.001	18	147, 176+, *K*, 176+, *K*, 176+, *K*, 176+, *K*		92, 120, 122, 122	113.5
Promin (salt) Subcutaneous	0.5	6	0.25	17	28, 29, 42, 45, 147	58.2	14, 14, 21, 23, 28, 31, 33, 39, 56, 107, 151	46.7
	0.5	15	0.001	17	75, 77, 133, 159	111.0	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
	0.5	28	0.001	18	111,* 138+, 151+, 165+, 176+, *K*		92, 120, 122, 122	113.5
Rodilone Oral	1.0	4	1.0	17	49+, 112+	80.5	25, 25, 25, 27, 42, 42	31.0
	1.0	5	1.0	33	21, 21, 22, 22, 26, 28+, 30+, 30+	24.0	18, 19, 21, 21, 21, 23, 25, 26, 26, 27	22.7
	1.0	5	0.25	14	28, 37, 39, 39, 40, 44, 154+	54.4	14, 16, 16, 29, 35, 37, 40, 43, 49	30.7
	0.5	6	0.103	36	68, 71, 82, 133	88.5	47, 65, 84, 161	89.2
	0.5	6	0.01	30	78, 88, 96, 145, 149	111.2	61, 75, 126, 144, 203	121.8
	0.5	9	0.001	18	119, 128+, 169+, *K* 169+, *K*, 169+, *K*		92, 120, 122, 122	113.5
	0.5	15	0.001	17	56, 57, 76, 77, 170 *K*		41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups, in which no averages are given.*

TABLE VIII

THERAPEUTIC DATA ON GUINEA PIGS

Series 7 (Miscellaneous agents)

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 59 Base Oral	0.5	10	0.001	10	46, 130 *K*		46, 130 *K*, 130 *K*, 130 *K*	
No. 60 Base Oral	0.5	10	0.001	10	44, 46	45.0	46, 130 *K*, 130 *K*, 130 *K*	
No. 62 Base	0.05	6	0.1	13	50, 133+, *K*		29, 64, 105, 114	78.0
Oral	0.05	10	0.001	10	45, 45	45.0	46, 130 *K*, 130 *K*, 130 *K*	
No. 63 Base	0.125	6	0.1	13	56, 69	62.5	29, 64, 105, 114	78.0
Oral	0.05	10	0.001	10	45, 46	45.5	46, 130 *K*, 130 *K*, 130 *K*	
No. 64 HCl Oral	0.05	6	0.1	13	24, 88	56.0	29, 64, 105, 114	78.0
No. 65 Base	0.03	10	0.001	10	46, 47	46.5	46, 130 *K*, 130 *K*, 130 *K*	
No. 66 HCl	0.025	6	0.1	13	45, 133+, *K*		29, 64, 105, 114	78.0
Oral	0.025	8	0.001	10	77	77.0	46, 130 *K*, 130 *K*, 130 *K*	
No. 66 HCl	0.0125	6	0.1	13	36, 44	40.0	29, 64, 105, 114	78.0
Subcutaneous	0.0125	10	0.001	10	130 *K*, 130 *K*		46, 130 *K*, 130 *K*, 130 *K*	
No. 67 Na salt	0.125	6	0.1	13	60, 102	81.0	29, 64, 105, 114	78.0
Oral	0.125	10	0.001	10	82, 130 *K*		46, 130 *K*, 130 *K*, 130 *K*	
No. 68 Base	0.125	6	0.1	13	60, 133+	96.5	29, 64, 105, 114	78.0
Oral	0.125	10	0.001	10	46, 77	61.5	46, 130 *K*, 130 *K*, 130 *K*	
No. 75 Oral	0.1	4	0.5	14	25, 25	25.0	21, 25, 26	24.0
No. 75 Subcutaneous	0.013	5	0.5	14	35, 36	35.5	33, 33, 63, 66	48.7
No. 76 Oral	0.03	5	0.5	14	46	46.0	33, 33, 63, 66	48.7
No. 76 Subcutaneous	0.03	5	0.5	14	36, 39	37.5	33, 33, 63, 66	48.7
No. 77	0.10	5	0.5		36	36.0	33, 33, 63, 66	48.7
Oral	0.05	4	0.5	14	21, 22	21.5	21, 25, 26	24.0
No. 77 Subcutaneous	0.03	5	0.5	14	65, 70+	67.5	33, 33, 63, 66	48.7
No. 78 HCl	0.25	4	1.0	17	27	27.0	25, 25, 25, 27, 42, 42	31.0
Oral	0.25	4	1.0	15	35	35.0	39, 40, 51	43.3

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups—no averages given.*

TABLE VIII—*Concluded*

THERAPEUTIC DATA ON GUINEA PIGS—*Concluded*

Series 7 (Miscellaneous agents)—*Concluded*

Compound and administration	Treatment		Culture of *T.B.* employed for inoculation		Duration of survival of treated animals		Duration of survival of untreated controls	
	Dosage, mgm./gm.	Days	Dried wt., mgm.	Age, days	Days	Average, days	Days	Average, days
No. 78 HCl Subcutaneous	0.04	4	1.0	17	39, 44	41.5	25, 25, 25, 27, 42, 42	31.0
Sulphapyridine Oral	1.0	10	0.25	15	47, 48, 49, 52, 54, 82, 83	59.3	31, 36, 40, 49, 50, 109, 124, 131, 142, 162	87.9
	1.0	7	0.001	31	212+, *K*, 212+, *K*, 212+, *K*, 212+, *K*		106, 126, 141, 203, 210	157.2
	1.0	15	0.001	17	49, 54, 113, 161, 161	107.6	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
Sodium sulphapyridine Subcutaneous	0.25	4	0.5	14	19, 22	20.5	21, 25, 26	24.0
	0.50	3	1.0	17	51	51.0	25, 25, 25, 27, 42, 42	31.0
Sulphathiazole Oral	0.5	5	0.5	15	27, 35	31.0	35, 35, 35, 38	35.7
	0.75	6	0.25	11	35, 136+	85.5	21, 23, 38, 120	50.4
	0.75	10	0.10	21	21, 28, 33, 56+, 128+	52.5	21, 28, 29, 32, 32, 35, 35, 35, 36, 46	32.9
	0.75	7	0.001	31	127, 148, 170, 186, 212+, *K*		106, 126, 141, 203, 210	157.2
	0.75	15	0.001	17	39, 66, 68, 71	61.0	41, 53, 57, 60, 64, 84, 86, 119, 170 *K*, 170 *K*	
Sodium sulphathiazole Subcutaneous	0.5	6	0.25	11	23, 24	23.5	21, 23, 38, 120	50.4

NOTE: + = *Survived longer than all the untreated controls of the same series.*
K = *Killed to complete series of treated or control groups—no averages given.*

Discussion

The data presented in this paper need little discussion. As can be seen from the tables none of the agents tested appears to offer any promise as a cure for tuberculosis. The results obtained with a number of the compounds studied were nevertheless interesting from the experimental viewpoint; but even in these instances the data can be regarded only as suggestive. Further study of some of these or related substances might be worthwhile.

In connection with the data presented in Tables II to VIII, it should be stated that survival of one or more animals longer than all the corresponding untreated controls in a single experiment was observed on the first trial with a number of the agents tested. This observation was considered significant

however only when it could be repeated; as can be seen from the tables in several instances this was not the case and the compounds used were considered to be without action or negative.

Of the 22-*p*-aminobenzene derivatives tested (Table II), the following six:— *p*-aminophenol (No. 1), *p*-ethylaniline (No. 4), *p*-chloroaniline (No. 6), *p*-aminophenyl hexyl ether (No. 8*a*), ethyl-*p*-aminobenzoate (No. 44), and 2,4-dichloroaniline (No. 57) resulted, in more than one experiment, in some animals surviving for shorter or longer periods of time beyond the untreated controls. Furthermore, in many of these experiments such animals also survived longer than the animals that were treated at the same time with other agents.

Similar results were obtained with *p*-N-ethylaminophenol (No. 34), as shown in Table V, in which the data obtained from the study of a series of eight *p*-N-ethylaminobenzene derivatives are summarized.

The results obtained with the 10 isomeric hydroxychloroanilines studied (Table VI) were particularly interesting. Thus, some prolongation in survival was noted with four of the compounds, namely: 3-chloro-4-hydroxyaniline (No. 46), 2-chloro-4-hydroxyaniline (No. 47), 2-chloro-5-hydroxyaniline (No. 50), and 2-hydroxy-3-chloroaniline (No. 52).

Some results of a similar nature were also obtained with the two sulphone derivatives, promin and rodilone, (Table VII) and sulphathiazole (Table VIII).

Finally, it may be stated that none of the other compounds, related to the above agents (Tables II and V to VIII), or belonging to any of the other groups of substances studied (Tables III, IV, and VIII), showed any significant influence upon the duration of survival of the animals treated with them. The results obtained with these 65 compounds may therefore be considered entirely negative.

In Table IX is presented an analysis of the results obtained with those 14 agents that showed in two or more experiments some prolongation in the survival of animals. Thus, this type of result was observed in 28.6 to 77.7% of the experiments carried out with these agents, and the number of animals outliving all controls in the different series of experiments varied from 16.4 to 40%.

In contrast with the above findings are some data presented in the same tables and compiled from results obtained in all experiments performed with two other series of agents, namely, 10-*o*-aminobenzene compounds (Table III) and 11 *m*-aminobenzene compounds (Table IV). All of these agents were considered without effect. Indeed, no compound in these two groups showed more than one experiment in which animals outlived their controls, and this effect was only observed in 6.9 and 10.7% respectively, of all the experiments. Furthermore, there were only 3.4 and 5.0% respectively, of animals in the two series that survived longer than the untreated controls.

TABLE IX

AN ANALYSIS OF THE RESULTS OBTAINED WITH THOSE AGENTS SHOWING PROLONGATION IN THE SURVIVAL TIME OF SOME OF THE ANIMALS TREATED WITH THEM

Compound No. or name	No. of different series of experiments performed	No. of different series in which animals lived longer than in controls	Total No. of animals treated	Total No. of animals outliving all corresponding controls
1	13	6 (46.1%)	61	10 (16.4%)
4	8	6 (75.0%)	27	10 (37.0%)
6	27	17 (63.0%)	172	32 (18.6%)
8*a*	9	4 (44.4%)	31	8 (25.8%)
44	9	7 (77.7%)	30	9 (30.0%)
57	4	2 (50.0%)	16	5 (40.0%)
34	6	4 (66.6%)	20	8 (27.2%)
46	4	2 (50.0%)	11	3 (27.2%)
47	8	3 (37.5%)	28	5 (17.8%)
50	18	11 (61.1%)	92	20 (21.7%)
52	7	2 (28.6%)	25	3 (12.0%)
Promin	11	4 (36.3%)	60	10 (16.6%)
Rodilone	7	4 (57.1%)	36	10 (27.7%)
Sulphathiazole	6	3 (50.0%)	20	4 (20.0%)
Series II (10 *o*-aminobenzene compounds as shown in Table IV)	28	3 (10.7%)	59	3 (5.0%)
Series III (11 *m*-aminobenzene compounds as shown in Table V)	29	2 (6.9%)	58	2 (3.4%)

In conclusion, it must be stated that none of the agents tested showed any definite evidence of curative action, since in all experiments gross evidence of tuberculosis could still be detected in all treated as well as untreated animals no matter how long they survived.

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